

Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*

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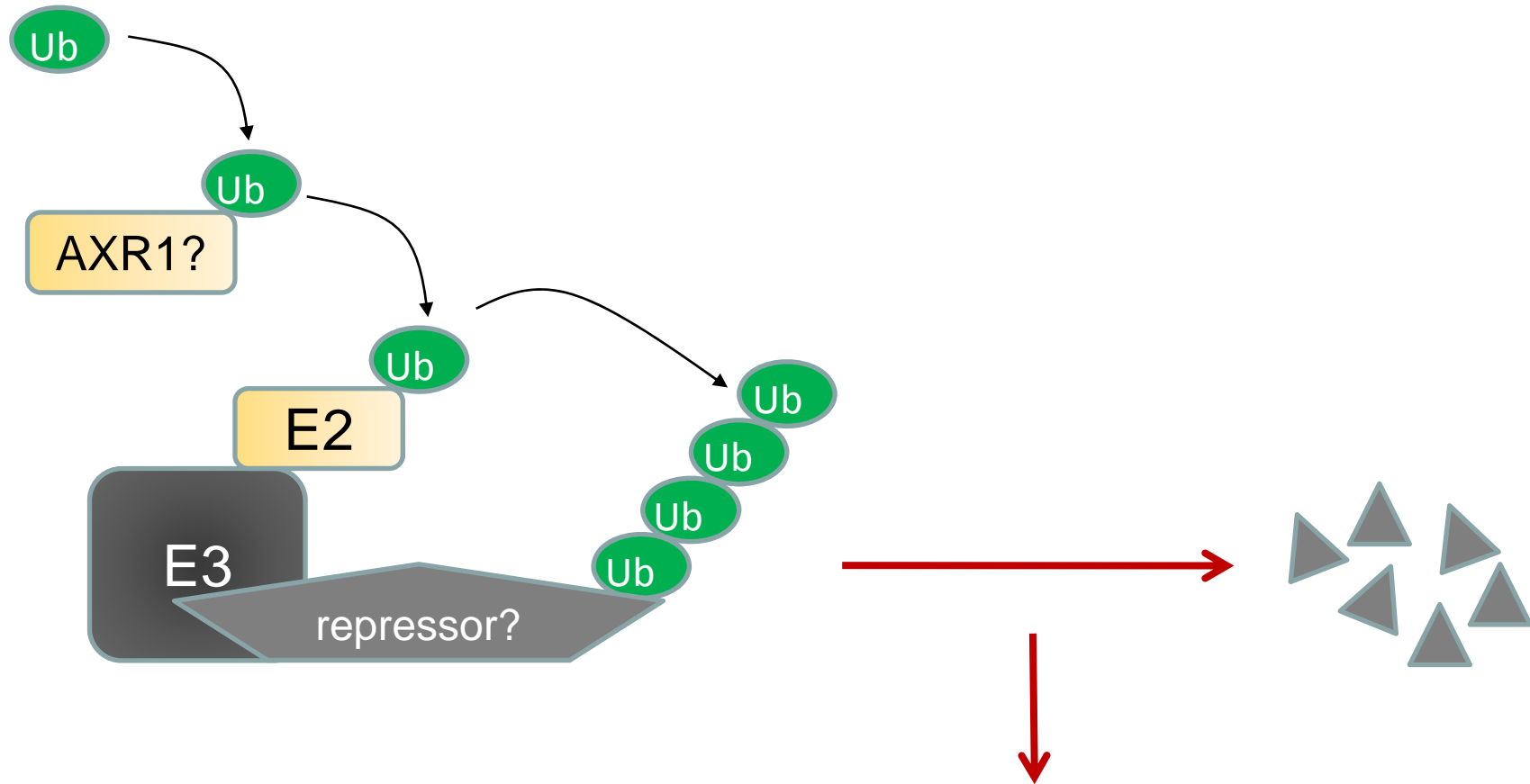
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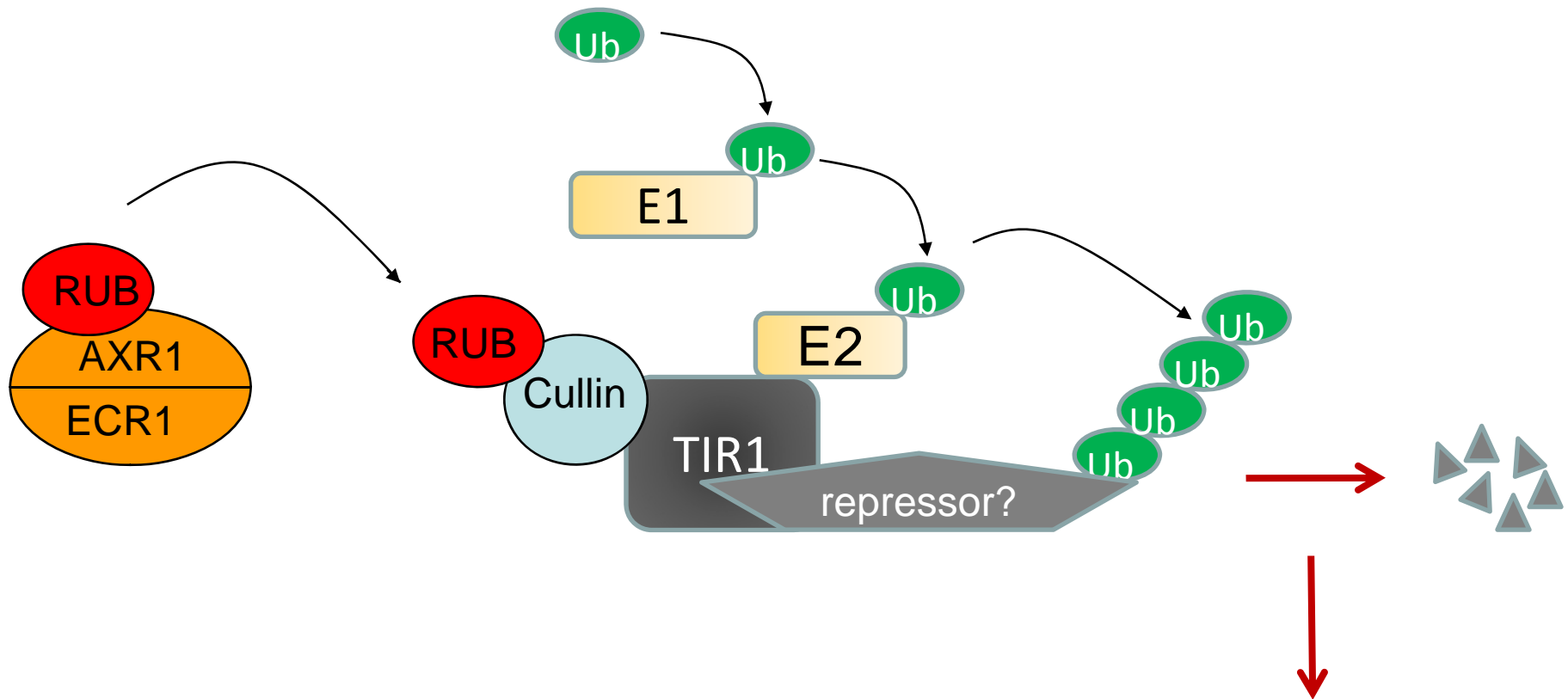
Mandy Conrad

Bisheriges Modell



AXR1 similar to ubiquitin-activating enzyme E1

auxin response



~~AXR1 similar to ubiquitin-activating enzyme E1~~

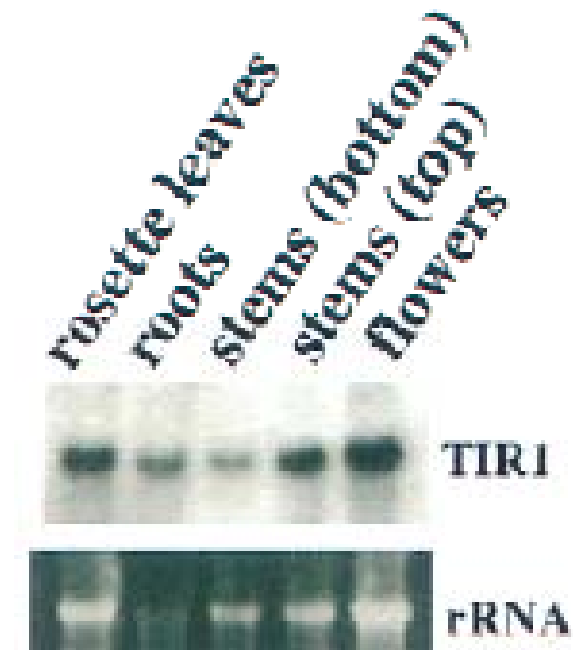
auxin response

Kernaussagen dieses Papers

- Beweis, dass *TIR1* mit *ASK1* & *ASK2* (=Skp-like) interagiert
- Mutationen im *ASK1*-Gen → verminderte Auxin-Antwort
- Übermäßige Expression von *TIR1* → morphologische Unterschiede zum WT
- neue Funde weisen darauf hin, dass SCF^{TIR1} - Modell realistisch ist
- → Auxin-Antwort = SCF-vermittelt

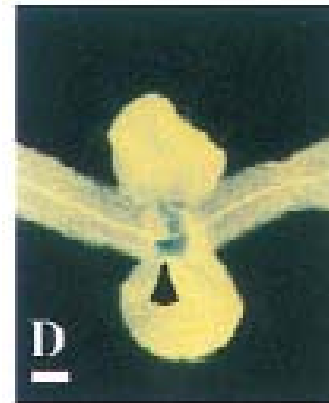
Analyse der TIR1-Expression

Northern Blot



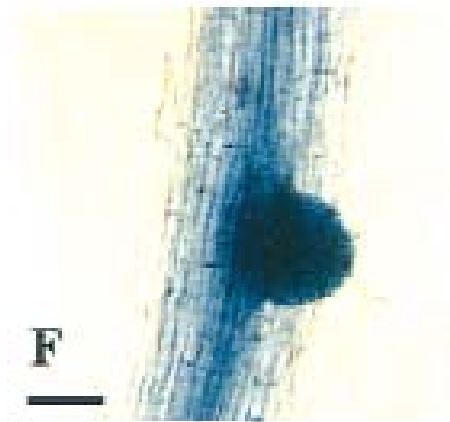
Nachweis der TIR1-Expression

Färbung durch GUS-Reporter



Nachweis der TIR1-Expression

Färbung durch GUS-Reporter



In-situ-Hybridisierung

...TCAT...

RNA

...AGTA...

Antisense-RNA (markiert)

Durch künstlich hergestellte Sonde Komplementarität der Stränge
→ Blaufärbung

...TCAT...

Sense-RNA

Sense-RNA bindet durch fehlende Komplementarität nicht an RNA
→ keine Färbung

Nachweis der TIR1-Expression

In-situ-Hybridisierung



Antisense



Sense



Nachweis der TIR1-Expression

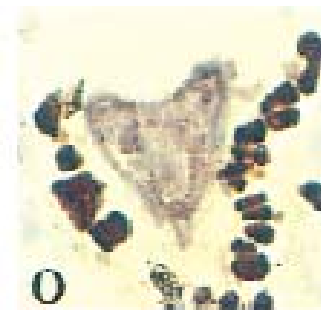
In-situ-Hybridisierung



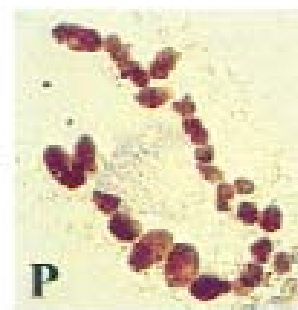
Antisense



Sense

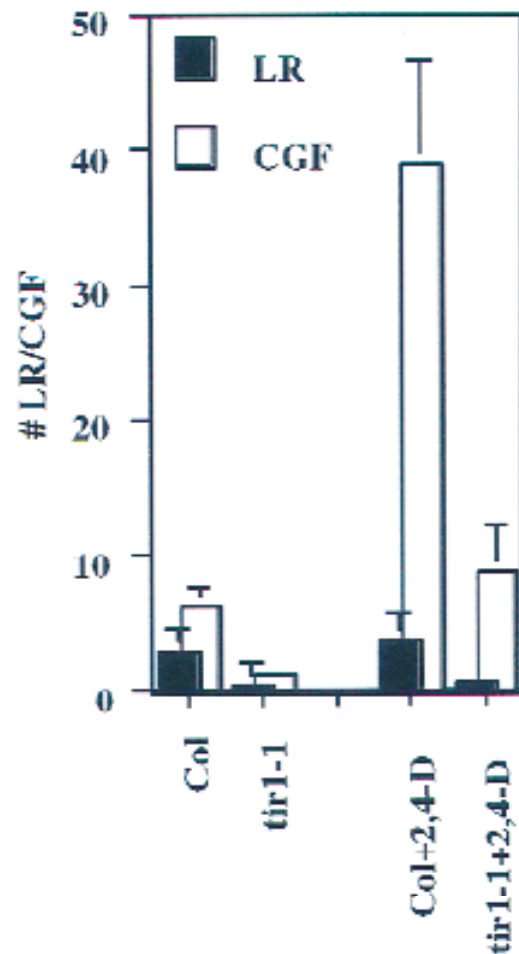


Antisense



Sense

Auswirkungen von Auxinbehandlung

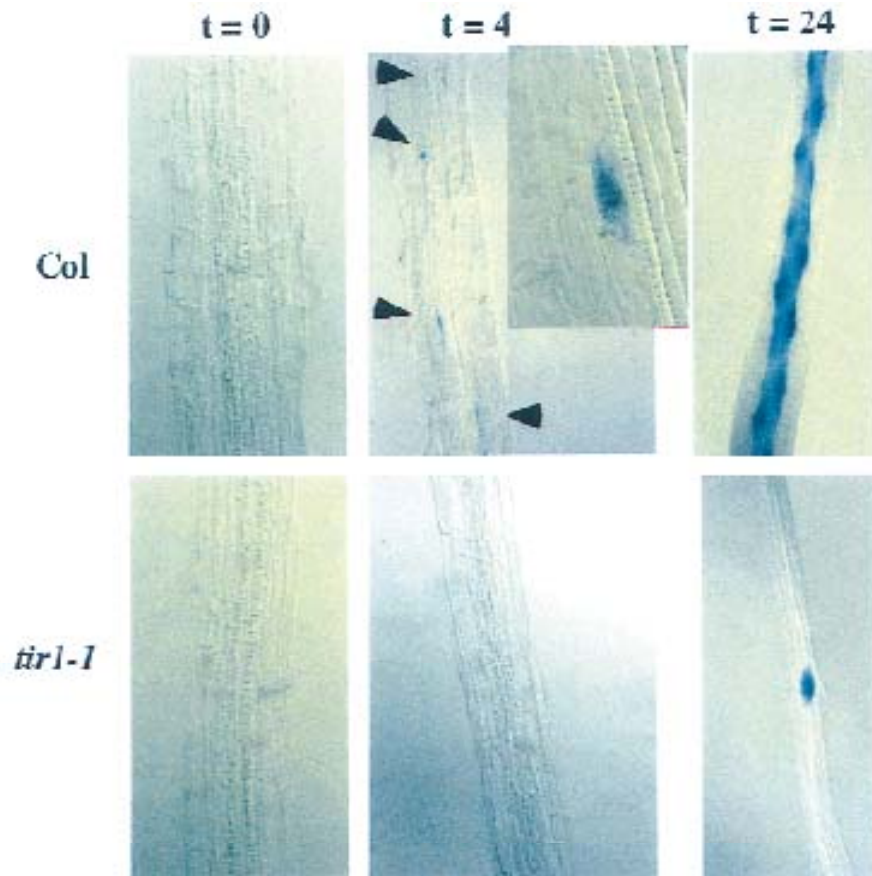


LR – lateral roots

CGF – Cyc1At-GUS-Foci

TIR1-Expression geschieht bevor
Cyc1At exprimiert wird

Auswirkungen von Auxinbehandlung auf Lateralwurzeln



**TIR1 notwendig für Teilung
von Perizykelzellen**

Identifikation der mit TIR1 interagierenden Proteine

```

ASK2 -----
ASK1 -----
HsSkp1 -----
ScSkp1 NVTSNVVLYS GGGGFTVVK KIAKRLLLK NYLNDHEDSN LKSSDGEFPE

ASK2 LDEAVLESQ TIRHNS... ..DDHDDH PLPNVESEIL SSVIEWGRRH
ASK1 VDEAVLESQ TIRHNS... ..DDHDDH PLPNVESEIL ASVIEWGRRH
HsSkp1 VDVAESQGV TIRHNSDGLG MDDHDDH PLPNVESEIL ASVIEWGRRH
ScSkp1 SDSDHNSK INNNGDDH MDDHDDH PLPNVESEIL ASVIEWGRRH

ASK2 VGRACKSTT ADRAATTT TVASGSDSD LKNDGEFPE VDSILFELI
ASK1 VGRACKARA VGRRA... ..SDSD LKNDGEFPE VDSILFELI
HsSkp1 KDDPPFD... ..SDSD LKNDGEFPE VDSILFELI
ScSkp1 KDDPPFD... ..SDSD LKNDGEFPE VDSILFELI

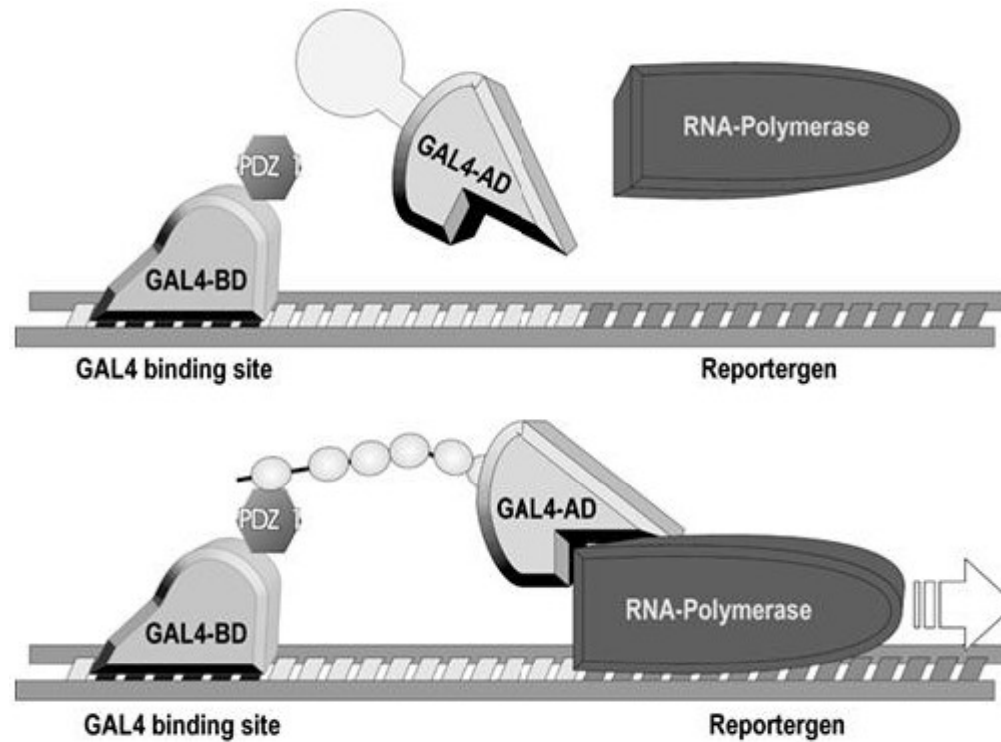
ASK2 LAANYLNER LLDLTGQTV DMKSTPEE IRTTFNND FTPEKSKK
ASK1 LAANYLNER LLDLTGQTV DMKSTPEE IRTTFNND FTPEKSKK
HsSkp1 LAANYLNER LLDLTGQTV DMKSTPEE IRTTFNND FTPEKSKK
ScSkp1 LAANYLNER LLDLTGQTV DMKSTPEE IRTTFNND FTPEKSKK

ASK2 RENQAF*
ASK1 RENQAF*
HsSkp1 RENQAF*
ScSkp1 RENQAF*

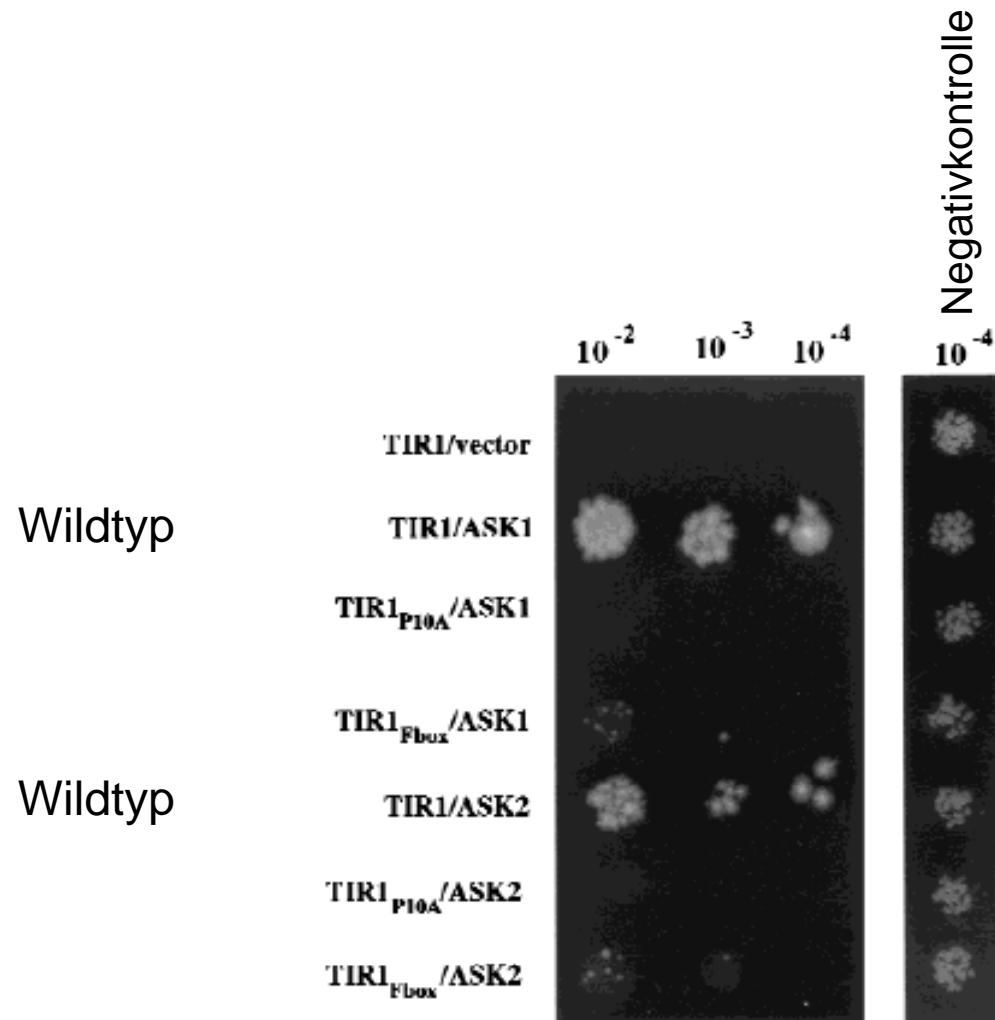
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Homologien von ASK1
und ASK2 zu Proteinen in
Mensch und Hefe

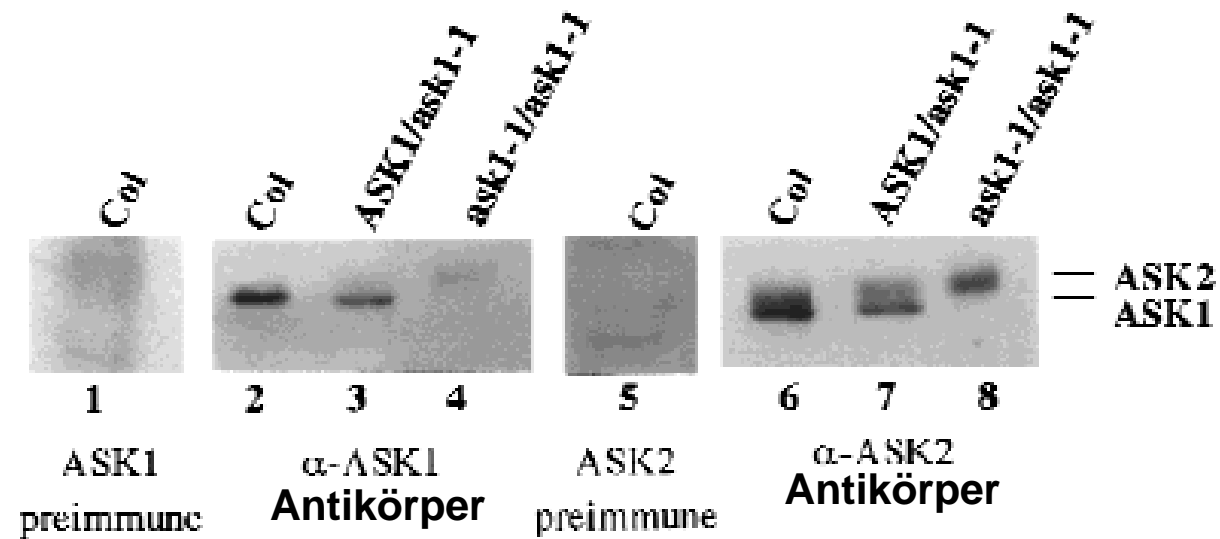
yeast two-hybrid System



Kontrolle in Hefe

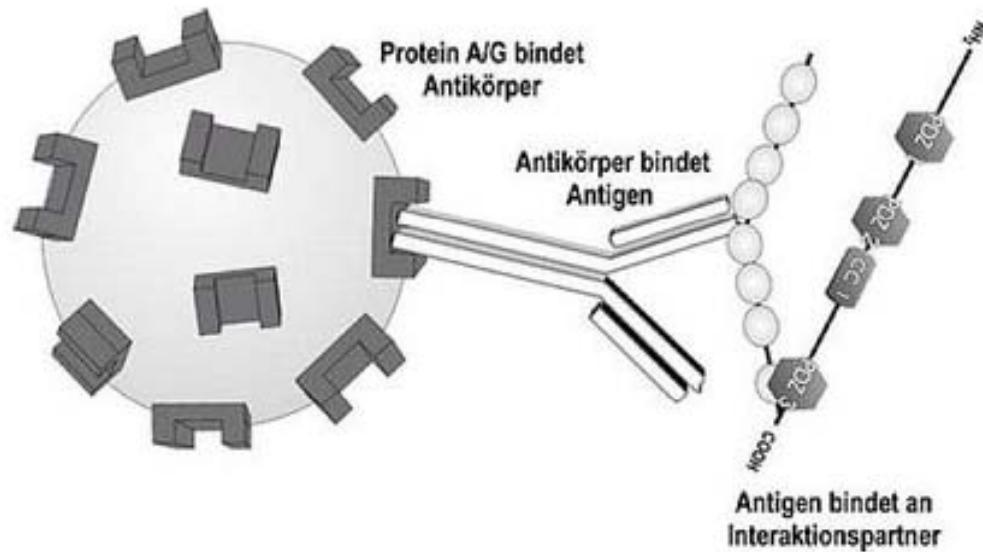


Western Blot



Ladekontrolle fehlt

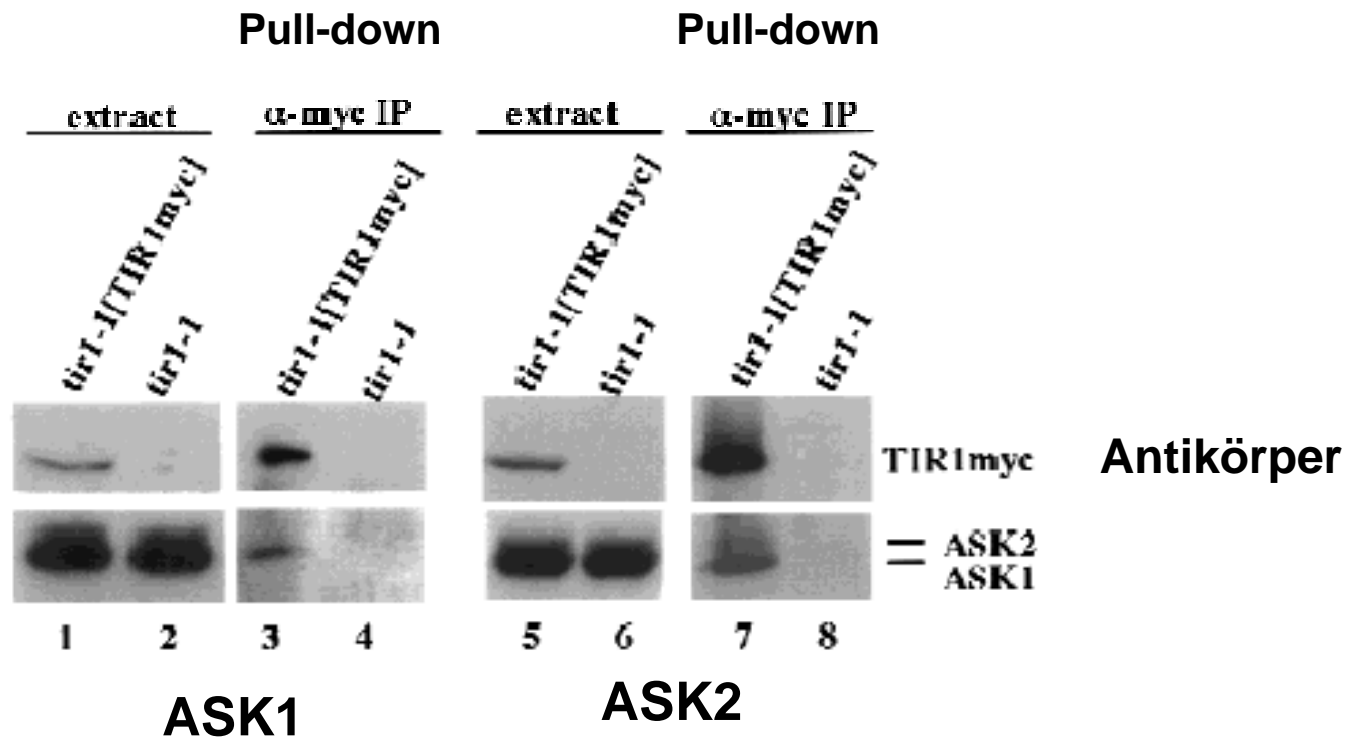
Co-Immunopräzipitation



**Nachweis von
Protein-Protein-
Wechselwirkungen**

Pull-down

Co-Immunopräzipitation

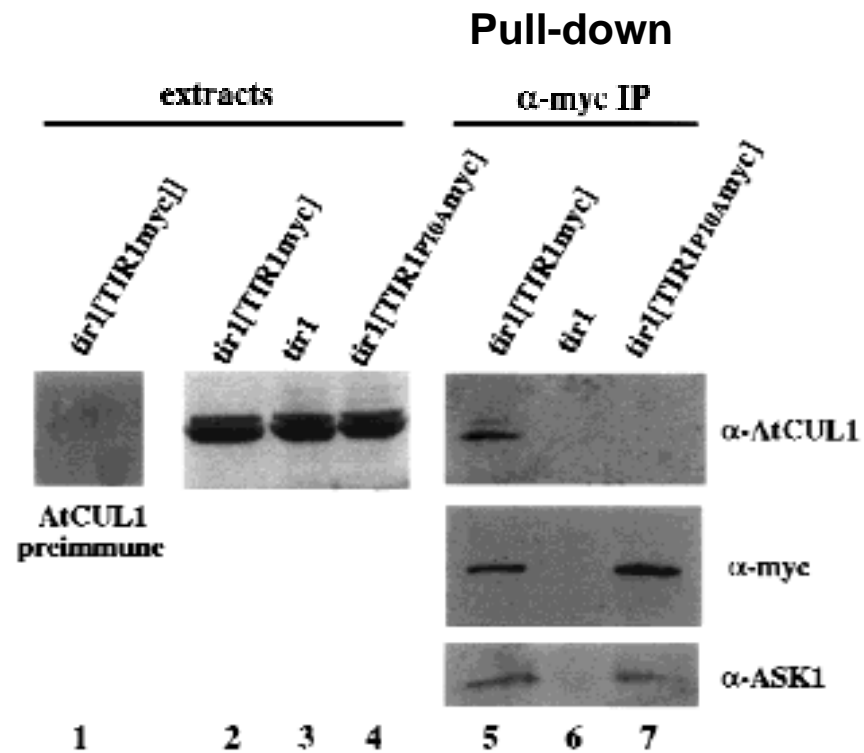


Identifikation von AtCul1

AtCull1	-----	-----	MEKFTIDLSQ	QNDYMQTQIT	AKRILEGSLQ
EsCul-4A	-----	-----	QINQGLGSGK	HQASTQKQVY	SELOPTWYK
Cdc53	MSSTLPSSGQ	LEATWRIET			
AtCull1	EPAPDSQHN	KLITTYIYNG	KLKPFHDSIQ	QLYDKYRPAF	EDINSTVIF
EsCul-4A	-----	-----	EDSRGGQSTI	LVASRIYKRL	KNKLRKYHJL
Cdc53	VYETAIYNYCV	NKRSRSGHPS			
AtCull1	ALRKHEDPM	ELRLPNSN	HKVGVNLSR	FSYLMRYFIARIS
EsCul-4A	-----	-----	FTGALNEN	AFYVNYNYIV	QKRSDDGN
Cdc53	NFKQSHSDF	LPYVYVGR			
AtCull1	LPPIEDVPT	CFEDLVNKL	HSKWQAVIA	LYKSDPESQ	EDALLANVL
EsCul-4A	-----	-----	KDVLCEELD	QVQLREGI	QDNEISTAI
Cdc53	IFDVLGLCN	TKRNVDPDS			
AtCull1	DIYVDS.QGR	YSDPESPM	QDSSTYSSK	ASSWIDEDC
EsCul-4A	-----	-----	YVGVCKPV	KNQSLAAE	QQLKQSRV
Cdc53	ESLTAEDS	QDLKLEHNV			
AtCull1	PTLSDSDC	AKQBRVAV	YLSHSEPR	YKQSHRSL	VVASQLDSE
EsCul-4A	PTLSEVSKR	EDQVQVQ	YLDSETPKL	IAQVPSLCC	SHLSAILCL
Cdc53	TKYTFKAGI	KKRQKCAVI	YVQDCKPL	SMALVPLAT	SH.....DEKL
AtCull1	HSRCLGSD	EDVQDSRY	RPKRLRL	KPVNLSKQ	ATAC.....
EsCul-4A	CLDEGLD	NWVDFQHY	QVPSVRCG	QALLQMSY	KRSC.....
Cdc53	ENKPVVGLA	EDKRTISL	ALHSDPLI	FRSRYGKY	QKQKQNEIS
AtCull1	NALVQSDV	ANQVFN...TSVQ	SVLDVSLD	SHDKQVYV
EsCul-4A	TSVQSD...KK	SKDQVLE	PKQVNVYV
Cdc53	SLAKSHKHI	NQDSEAPK	LALNTSES	PRDQGLLE	SHDPSKIP
AtCull1	EDQNYTLN	ALKRPAVDS	CG.....SVFSS	ALLAATPCN
EsCul-4A	VQPKHSDV	NKQVQVQ	IN.....KPRD	AKLQNVIS
Cdc53	EDKSDIPLA	HALDQVDS	INNFALPA	GSFKSQRSE	SEKLARQSI
AtCull1	HLRQSSKL	SDAANTLS	QVYVLAYIS	DKDPAHYT	SLALSLDQ
EsCul-4A	HLKQKPEA	SDSMD...	EDLHININ	OKDPAHYT	SLAKRLIN
Cdc53	ELKQKPEV	ASMSD...S	EDLHININ	OKDPAHYT	SLAKRLIN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
Cdc53	TSQSDN	SLSLQSDN	MSVSDN	PDQVSDS	EDQVSDN
AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
EsCul-4A	SHAVVSDS	SLSLQSDN	AAVSDN	PDQVSDS	NVSPQSDN
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AtCull1	SHANDSD	SLSLQSDN	QPTNNDN	VQDPAHYN	QNSQVYV
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Cdc53	TSQSDN				

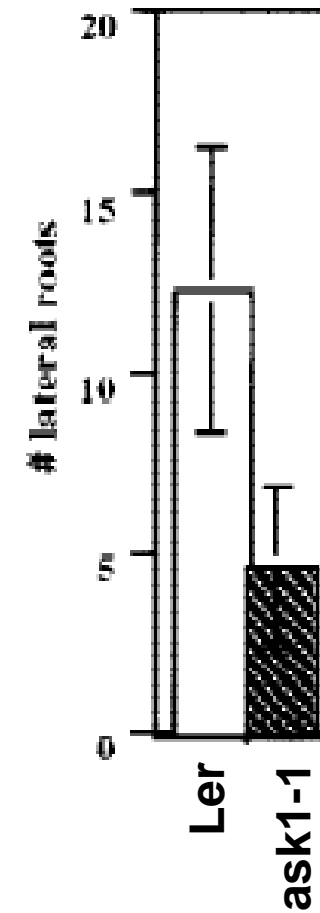
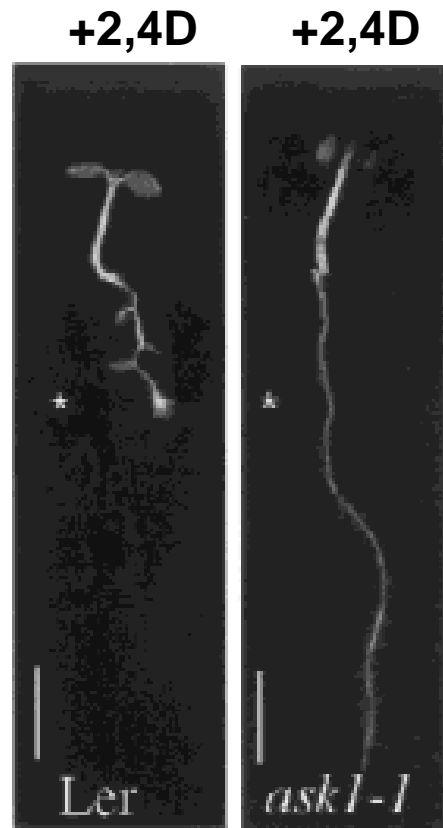
AtCUL1 stark homolog zum menschlichen Cul-4A und zum Cdc53 in Hefe

Pull-down für Cul1

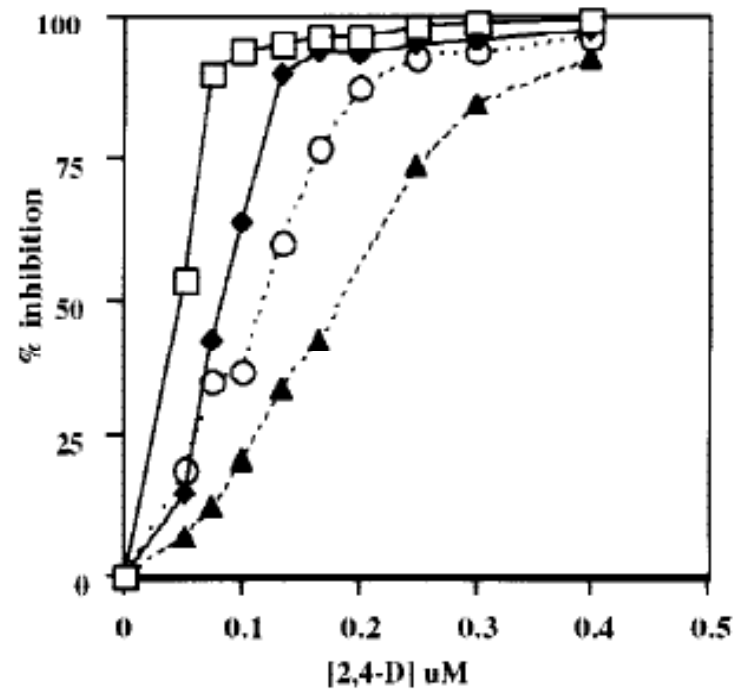


Antikörper

ASK1 für Auxin-response notwendig

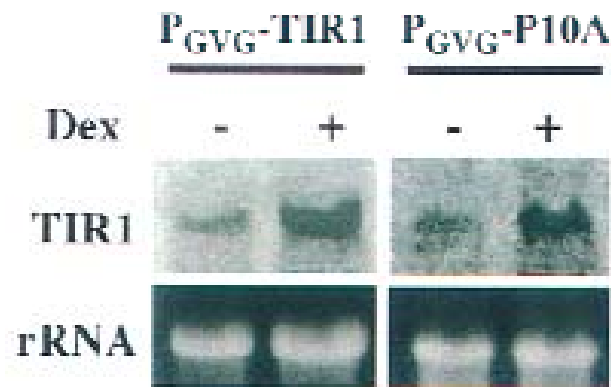


Wachstumshemmung durch Auxin



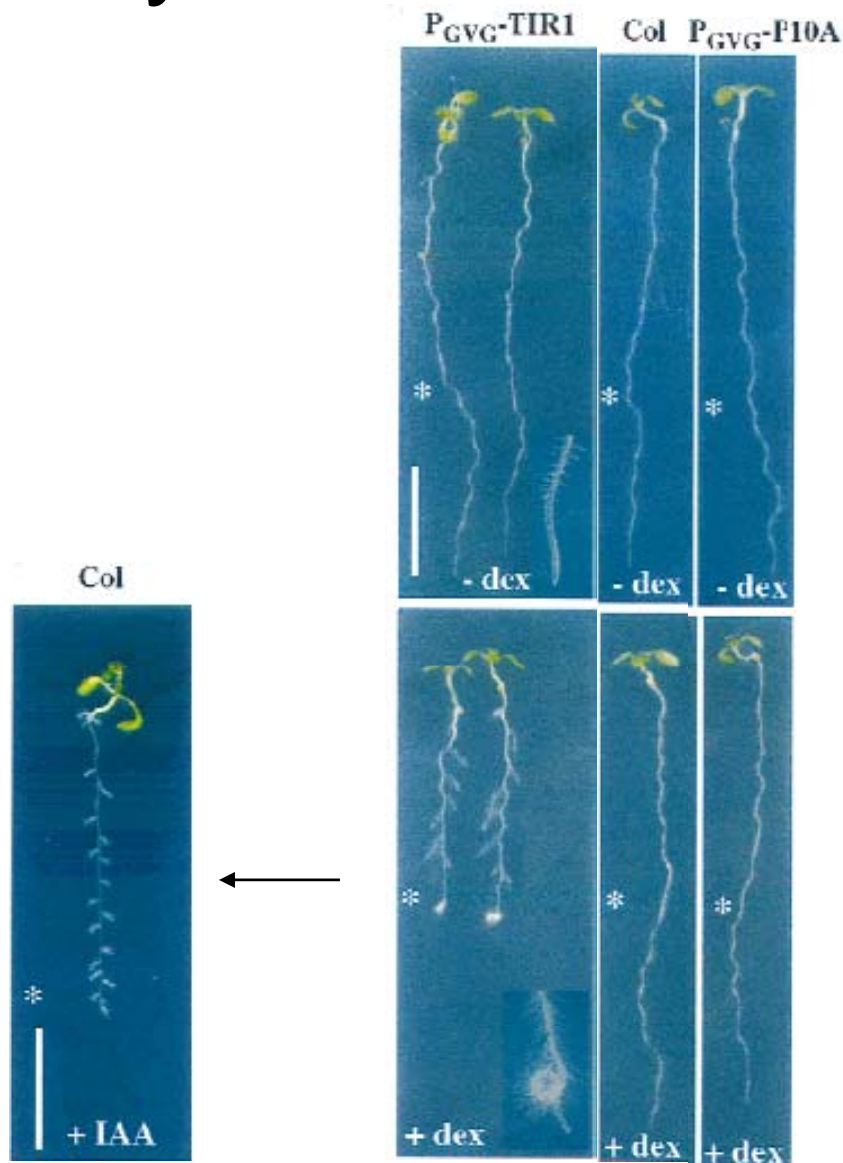
- Wildtyp
- tir1-1
- ask1-1
- ▲ tir1-1/ask1-1 (Doppelmutante)

Northern Blot für Dex-Promotor



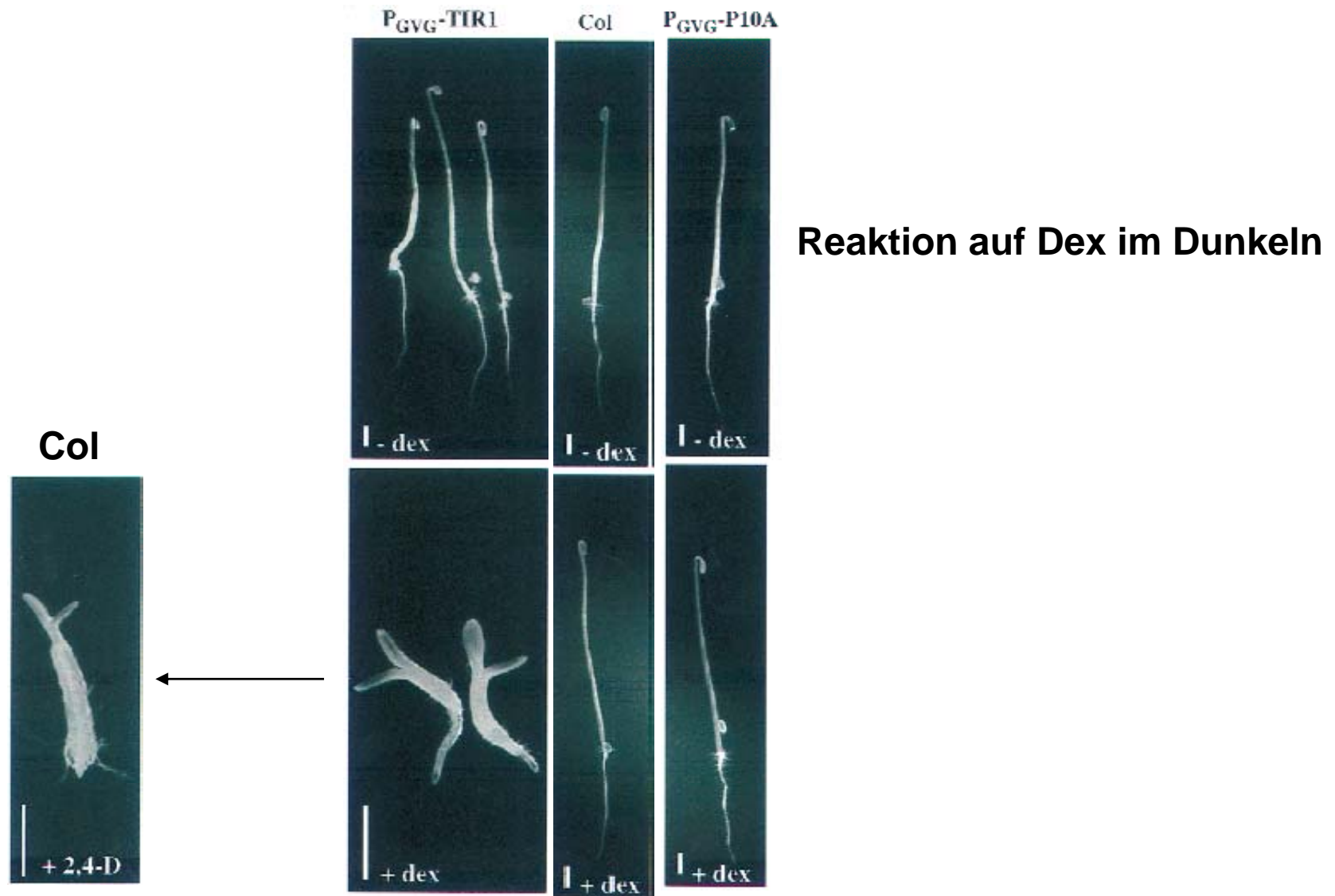
Bei Vorhandensein von Dex
und Promotor (P_{G_{VG}}) →
Überexpression von TIR1

Analyse der TIR1-Überexpression



Reaktion auf Dex im Licht

Analyse der TIR1-Überexpression



Modell von 1999 (Gray et al.)

